

# Toxicity of Culture Material of *Fusarium verticillioides* Strain MRC 826 to Nonhuman Primates

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We conducted a chronic feeding study in vervet monkeys (*Cercopithecus aethiops*) over 13.5 years. The experimental design consisted of two dietary treatment groups, each including males and females, fed varying levels of culture material of *Fusarium verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* Sheldon) strain MRC 826 mixed into their daily food ration. Two females were included as treatment controls. We conducted blood chemical analyses bimonthly and recorded all clinical signs during the course of the experiment. We took liver biopsies at various stages during the initial phase of the experiment. Several monkeys were terminated *in extremis* during the experiment. Detailed feed intake profiles were determined 5 years after the experiment began, and the fumonisin B (FB) mycotoxin content of the feed was determined during the final stages of the experiment. The apparent FB consumption patterns were related to changes observed in the biochemical parameters in the blood and urine, including the liver function enzymes and creatinine clearance as well as differential blood counts and sphingolipid levels in the serum and urine. An apparent no-effect threshold for kidney and liver damage is estimated to be between 0.11 and 0.18 mg FB/kg body weight (bw)/day, which corresponds to a feed contamination level of between 8.21 and 13.25 mg FB/kg bw diet. Apart from the effects on the liver and kidney, a wide variety of parameters, including cholesterol and creatine kinase, were also adversely affected. Several blood parameters, including white and red blood cells, also significantly decreased in the treated animals. The serum sphinganine level and the sphingosine/sphinganine ratio, monitored toward the end of the experiment, significantly increased in both the low-dose and high-dose animals. The present study provides important information about the diversity of lesions induced by culture material of *F. verticillioides* in vervet monkeys and the dosage levels of fumonisins to be used in long-term studies in nonhuman primates. **Key words:** culture material, fumonisins, *Fusarium verticillioides*, hepatotoxicity, nonhuman primates. — *Environ Health Perspect* 109(suppl 2):267–276 (2001). <http://ehpnet1.niehs.nih.gov/docs/2001/suppl-2/267-276gelderblom/abstract.html>

Toxicologic studies with culture material of *Fusarium verticillioides* (= *F. moniliforme*) grown on corn have been performed in a variety of experimental animals, including pigs, sheep, horses, baboons, and rats (1). The scientific importance of this fungal species, however, was first recognized with the finding that contamination of feedstuff, particularly corn, with *F. verticillioides* was responsible for natural outbreaks of the mycotoxicosis equine leukoencephalomalacia (ELEM) (2,3). This implied that the causative principle(s) occur(s) under natural conditions and could affect the health of livestock and also humans. The latter was of particular interest because many human populations in southern Africa consume corn as the major dietary staple (4). Efforts were launched in South Africa and elsewhere to develop sensitive screening methods to aid in the chemical isolation of the active compound(s). A duckling toxicity assay was designed whereby numerous isolates of the *F. verticillioides* were screened for the presence of toxic secondary metabolites produced by the fungus (5). Several isolates with different degrees of duckling toxicity were identified and formed the basis of further toxicologic studies in various animal species.

One of these, designated *F. verticillioides* strain MRC 826, was identified as one of the most toxic isolates from corn. This strain caused many toxic effects in experimental animals, such as ELEM in horses, pulmonary edema in pigs, acute nephrosis and hepatosis in sheep, and cirrhosis, intraventricular cardiac thrombosis, and nephrosis in rats (1). A less toxic strain, *F. verticillioides* MRC 602, also induced acute congested heart failure in baboons. A remarkable finding of these early experiments was that the main target organ differed in each animal species although certain organs, including the liver and kidneys, were consistently affected. It was suggested that rats provided the best screening system able to mimic biologic effects of the fungus in the various mammalian species (5). Toxicologic studies in rats eventually formed the basis for evaluating the carcinogenic effects of the potent mutagen fusarin C and subsequently for isolating the fumonisin (F)B mycotoxins from culture material of *F. verticillioides* MRC 826 (6,7).

Studies in nonhuman primates were initially performed with baboons (1). A prominent lesion was acute congestive heart failure, and liver cirrhosis was observed as a principal lesion in another baboon. The latter

study was performed between 5 months and 2 years at varying dietary levels of the culture material of *F. verticillioides* strain MRC 602. Subsequent studies were conducted in rats to evaluate the long-term toxicologic effects of different isolates of *F. verticillioides* including strain MRC 826 (8,9). At the same time, a long-term toxicity study was initiated at the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), Tygerberg, South Africa, in vervet monkeys (10,11) by feeding different dietary levels of *F. verticillioides* strain MRC 826. In this article we describe the clinical and biochemical data and dose–response effects in relation to the FB exposure levels during the course of the experiment (approximately 13.5 years).

## Materials and Methods

### Animals and Diets

Twelve vervet monkeys (*Cercopithecus aethiops*), consisting of eight females and four males weighing between 1 and 2 kg, were selected from the breeding colony of the MRC Primate Unit of the Experimental Biology Programme of the Medical Research Council, Tygerberg, South Africa. They were caged singly in a closed environment (26 ± 1°C) with a 12-hr photoperiod, 50% humidity, and 20 air changes/hr. The vervet monkeys were divided into three groups consisting of six animals (four females and two males) in the high-dose group, four animals (two males and two females) in the low-dose group, and two animals (females) in the control group. The diet consisted of 57 g of commercial pre-cooked corn meal, 1 g of an in-house vitamin mixture, and 10 g of a protein–vitamin–mineral supplement (PVM Product, Pretoria, South Africa). Culture material of *F. verticillioides* MRC 826 was incorporated at different

This article is based on a presentation at the International Conference on the Toxicology of Fumonisin held 28–30 June 1999 in Arlington, Virginia, USA.

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We thank N.P.J. Kriek for the preliminary histopathologic analyses and J.E. Fincham for his constructive criticism and involvement throughout this study.

Received 25 April 2000; accepted 27 September 2000.

concentrations during a pilot experiment to avoid excessive toxicity (Table 1). The control diet contained 0.5% autoclaved corn meal. All diets were prepared in bulk (approximately 9.5 kg) and stored at 4°C and from 1992 they were routinely analyzed for aflatoxins and FB mycotoxins. The mixed diet was prepared as a porridge after which vitamins D<sub>3</sub> (200 I.U.) and C (40 mg) were added before feeding. The estimated energy and nutrients supplied per vervet monkey per day have been calculated and published in detail elsewhere (11). The diet has been used to sustain successful in-house breeding over several generations with satisfactory development of the offspring (12). The diet was supplemented further with 35.4 ± 4.1 g of whole-wheat brown bread and a slice of apple (70.5 ± 6.6 g) in the afternoon. Feed wastage was monitored starting 4 years after the experiment began. This was accomplished by first weighing the dry and wet food as well as the wet wastage collected from underneath the cage. The dry wastage was extrapolated from the wet:dry ratio of the food. Housing the vervet monkeys in pairs after 5 years circumvented abnormal social behavior caused by solitary confinement. They also had access to large exercise cages for 24 hr/week.

One vervet monkey (female 681) of Group 1 was used in a pilot study (approximately 3 months) to evaluate early signs of toxicity from ingestion of culture material. As the experiment progressed, the level of the culture material incorporated in the diet was altered accordingly (Table 1) to avoid excessive toxicity. At that stage (1984/1985) the FB mycotoxins had not been characterized, and detailed chemical analyses of the diet and

culture material became possible only about 8 years after the experiment began (1991/1992). Subsamples of the different fungal culture batches used during the course of the experiment were retained and subsequently analyzed for fumonisins in order to calculate exposure levels. The different culture batches were prepared according to standardized procedures using autoclaved yellow corn kernels inoculated with single-conidial isolates of *F. verticillioides* strain MRC 826 and were incubated at 25°C for 2 weeks and then for 2 weeks at 15°C (8). Three different culture batches designated batch B (BB), batch 55 (B55), and batch 57 (B57) were used during the experiment. Batch BB was freeze dried; B55 and B57 were oven-dried at 45–50°C. The dry fungal cultures were ground to a fine meal and stored in airtight containers at 4°C.

### Clinical Monitoring

Appetite and habitus were observed daily; body weights, heart rates, and respiration rates were recorded at monthly intervals. Blood samples for measurement of clinical biochemical parameters were collected every 2 months throughout the experiment. These parameters included aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), gamma glutamyl-transferase (GGT), alkaline phosphatase (ALP), conjugated, unconjugated, and total bilirubin, glucose, urea, creatinine, sodium, potassium, chloride, calcium, phosphorous, and magnesium. These parameters reflect liver, kidney, muscle, respiratory, intestinal, and bone functions. Full and differential counts of venous blood, collected in potassium EDTA tubes, were obtained with a

Technicon H 6010 C cytochemical flow through cell counter (Beckman/Coulter, Cape Town, South Africa). Plasma lipids and lipoproteins were analyzed at the time the final liver biopsies were taken and when the animals were sacrificed.

Urinary creatinine clearance was monitored when increased serum urea and creatinine became evident. A 24-hr urine sample of each vervet was collected into a plastic container with the aid of a stainless steel funnel (13). The funnels drained the entire cage floor and a strainer prevented contamination with feces. Clearance of creatinine from the serum was expressed as milliliters per kilogram of body weight per minute.

### Disruption of Sphingolipid Metabolism

It has been demonstrated in a variety of animal species that exposure to fumonisin mycotoxins altered sphingolipid metabolism and that FB<sub>1</sub> and FB<sub>2</sub> inhibit the enzyme ceramide synthase in the sphingolipid biosynthetic pathway (14). At the cellular level this leads to an accumulation of sphinganine, which is then manifested in changes in the circulating levels of this base. During the course of the experiment, serum levels of the sphingoid bases sphingosine and sphinganine (and hence their ratio) were monitored over 60 weeks from January 1994 to March 1995 (15). By this time, the experimental groups (controls, low-dose and high-dose groups) consisted of two females each. Serum samples were drawn at regular intervals during this period to yield 10 sets of results. In addition, 24-hr urine samples were collected at the same time as the last two blood samples.

**Table 1.** Summary of the duration, dietary level, and daily intake profiles of different *F. verticillioides* MRC 826 culture batches used during the chronic feeding study in vervet monkeys.<sup>a</sup>

Group	No. of vervet (sex)	Feeding regimens						
		FCM <sup>b</sup> (MRC 826)	(%)	Commenced (dd/mm/yy)	Termination (dd/mm/yy)	Duration (days)	FMC intake (mg/kg bw/day)	Terminated
Group 1 High dose (trial)	681(F)	BB	5	13/12/84	03/01/85	21	705.8	30/06/87
		BB	1	04/01/85	18/01/85	14	141.2	
		BB	0.25	19/01/85	29/01/86	375	35.3	
		B55	0.50	30/01/86	31/03/87	425	70.6	
Group 1 High dose	688 (F) 702 (M) 705 (M) 709 (F) 712 (F)	BB	1	14/03/85	23/04/85	40	141.40	03/11/92
		BB	0.25	24/04/85	29/01/86	280	35.30	30/08/89
		BB	0.50	30/01/86	31/03/87	425	70.60	24/07/92
		B55	0.50	01/04/87	24/10/88	572	70.60	14/09/95
		B57	0.50	25/10/88	05/06/91	953	72.8 ± 17.9 <sup>c</sup>	11/11/98
		B57	1	06/09/91	11/11/98	2623	151.0 ± 36.8 <sup>c</sup>	
Group 2 Low dose	696 (M) 700 (M) 710 (F) 711 (F)	BB	0.25	14/03/85	23/04/85	40	34.0	14/01/94
		BB	0.1	24/04/85	29/01/86	280	14.0	30/10/92
		BB	0.25	30/01/86	31/03/87	425	34.0	30/07/97
		B55	0.25	01/04/87	24/10/88	572	34.0	11/11/98
		B57	0.25	25/10/88	05/06/91	953	35.2 ± 8.2 <sup>c</sup>	
		B57	0.5	06/09/91	11/11/98	2623	73.6 ± 17.4 <sup>c</sup>	
Group 3 Control	707 (F) 708 (F)	Corn meal	0.5%	14/03/85	11/11/98	4993	84.5 ± 15.7 <sup>c</sup>	11/11/98
		Corn meal	0.5%	14/03/85	11/11/98	4993	84.5 ± 15.7 <sup>c</sup>	11/11/98

<sup>a</sup>Values are means ± SD of at least 20 determinations per group of the surviving vervets. <sup>b</sup>Total fumonisin concentration: MRC 826 BB = 5.31 mg FB/kg, MRC 826 B55 = 2.61 mg FB/kg, and MRC 826 B57 = 3.43 mg FB/kg. <sup>c</sup>Calculated from feed intake data monitored 3–6 times per year from 1989.

## Biopsy and Necropsy Procedures

The toxic effects of the different feeding regimens were evaluated in liver biopsies taken at different time intervals during the course of the experiment. Subsequent investigations regarding histopathologic changes in the liver and kidneys of the animals terminated during the experiment and those sacrificed at the end of the experiment are currently in progress and will be reported elsewhere. Initial biopsies were taken on alternative monkeys of each group on days 2 and 6 of the experiment, and all individuals were subjected to biopsies at 180, 300, 520, 890, and 1,631 days after commencement. Wedge liver biopsies were taken by laparotomy under halothane general anesthesia. For the histopathologic evaluation of these biopsies, we used different staining procedures, including orcin stain to detect bile acids, cholesterol, and copper in hepatocytes; standard hematoxylin and eosin; periodic acid–Schiff; and reticulin stains (10,11).

Necropsy procedures in monkeys that were terminated *in extremis* and at the end of the experiment were performed after perfusion fixation under surgical anesthesia. For this fixation, 4% buffered formalin (pH 7.2) was perfused into the left ventricle at physiologic pressure (100 mm Hg) and flow. Before the perfusion, blood samples and fresh biopsies were collected from the liver and kidneys

for electron microscopy and biochemical analyses. Cutting the femoral and jugular veins as well as cutting deeply into the kidneys and liver ensured free flow of fixative. Immersion of the organs in the buffered formalin, at which time the organ weights were recorded, completed fixation.

## Data Analysis

All statistical analyses and graphical summaries were performed with the Number Cruncher Statistical System (NCSS 2000), Statistical System for Windows (16). The correlation coefficients reported are the partial coefficients—the correlation between two variables, with the effect of other possible confounders excluded. The *p* values reported are the partial values obtained from the multiple regression analyses—the relationship between the dependent and the independent variables after the effect of the other independent variables has been taken into account. The plots used the spline smoother of the scatterplot option of the program to draw the curves.

## Results

### Clinical Observations, Biopsies, Necropsies, and Termination

The biopsies, which were taken at stipulated time points, resulted in the death of one

female. The histopathologic evaluations of these biopsies have been reported elsewhere and suggested an active chronic toxic hepatitis (10) at a very early stage, whereas mild portal-to-portal fibrosis was reported in some animals of the high-dose and low-dose groups (11). Clinical observations indicated that the females were in better condition than the males. A number of vervet monkeys of the low-dose and high-dose groups were terminated during the course of the experiment because of general poor health (Table 2). These conditions of cachexia include ataxia, poor food consumption, and weight loss. The experiment was terminated 4,990 days after commencement. At that stage the two controls (females 707 and 708) and only one each of the low-dose (female 711) and high-dose (female 712) groups survived. The livers of all the animals killed during treatment and at termination showed signs of chronic toxic hepatitis. Some animals (females 711 and 712) showed patchy atheromatous plaques in the aorta. One animal (male 702) died from brain hemorrhages with lesions in the cerebellum, midbrain, and the right cortex. The kidneys were also affected, although some animals, such as male 702, showed no specific lesions. In one of the animals (male 705) terminated after 2,689 days of treatment, only microscopic cytoplasmic vacuolization of

**Table 2.** Clinical symptoms and lesions observed at different times during the experiment as well as the major macroscopic changes noticed at termination.<sup>a</sup>

Number	Sex	Dosage group	Days after commencement	Symptoms and/or lesions	Necropsy (macroscopic observations)
681	F	H	929	Hemorrhage in abdomen (terminated)	Pale liver with irregular surface
688	F	H	2,791	Alopecia on trunk and thighs	Fibrotic, atrophic liver (chronic toxic hepatitis)
702	M	H	1,630	Cachexic, ataxic, difficulty in handling and eating food	Hemorrhages of about 1 cm in grey matter of midbrain; most recent and largest lesions found in the cerebellum, midbrain, and right cortex of the midbrain
705	M	H	2,689	Focal hemorrhages in the skin	Necrosis of the distal and proximal rectum; fibrotic liver (chronic toxic hepatitis); hard kidneys with streaks of blood from the corticomedullary junction
709	F	H	2,497	Weight loss, yellow discoloration of the periorbital skin, blood in feces	Fibrotic, atrophic liver (chronic toxic hepatitis)
			3,836	Yellow discoloration of the skin	
712	F	H	4,990	Termination of experiment	Liver atrophic accentuated lobulation (chronic toxic hepatitis); multifocal to confluent atheromatous plaques throughout the aorta
696	M	L	2,414	Torsion of the colon	Small, fibrotic liver (chronic toxic hepatitis); colon and stomach contained hemorrhagic ulcers
			3,228	Torsion of the colon	
700	M	L	896	Mild subcutaneous hemorrhage in the groin area	Fibrotic liver (chronic toxic hepatitis); hemorrhagic diathesis; mild icterus
			1,762	Erythema on right thigh and left abdomen	
			2,723	Mild alopecia on left arm, blood in feces	
			2,747	Vomition, reluctant to move	
710	F	L	2,650	Starring coat	Hard fibrotic liver (chronic toxic hepatitis); kidneys showed petechial hemorrhages in cortex; enlarged lymph nodes and inflamed mesenteries
			2,738	Blood in feces	
			2,812	Yellowish discoloration of skin	
			4,521	Cachexia, vomition	
711	F	L	896	Focal hemorrhages in the skin of abdomen and left leg (disappeared after 30 days)	Atrophic liver with nodular appearance and accentuated lobulation and cobblestone effect; chronic toxic hepatitis; a few patchy atheromatous plaques in the aorta
			3,707	Consistently poor feed consumption	
			4,990	Terminated experiment	
707	F	Controls	4,990	Termination of experiment	No clinical signs
708	F	Controls	4,990	Termination of experiment	No clinical signs

<sup>a</sup>All cases were terminated *in extremis* except females 707, 708, 711, and 712.

epithelial cells of convoluted tubules was present. The other cases that were terminated demonstrated mild to severe cortical nephrosis manifested as toxic atrophy of the epithelia of the convoluted tubules and probably the glomeruli. No acute or chronic inflammation was present. Final body and organ weights (Table 3) indicated that the relative liver weights of the high-dose group appeared to be slightly lower than that of the low-dose group, but the low number of animals and differences in sex meant that statistical analyses could not be performed. However, despite the toxic effects encountered in the liver and kidneys, multiple regression analyses showed a positive correlation ( $r = 0.47$ ;  $p < 0.0001$ ) of fungal culture material (FCM) intake and body weight. It should be noted that the higher dose levels were administered from 1992 onward during the stage of the experiment where the vervets had established their adult weight. The loss in body weight in those vervet monkeys sacrificed *in extremis* was encountered only a few months before

termination. Therefore the dietary levels of FCM used apparently did not affect the normal body weight gain of the vervet monkeys with age.

### Feed and Fumonisin Intake Parameters

Feed intake profiles were conducted in detail about 4 years after commencement of the experiment. Discovery of the fumonisins (7) and the development of the relevant analytic methodology (17) resulted in routine analyses of the maintenance diet for the fumonisins, including retrospective analyses of the different culture batches used. Given these data, the FB intake during the earlier part of the experiment could be estimated fairly accurately (Table 4). However, several variables complicate the calculation of accurate exposure data, expressed as mean intake per kilogram body weight per day for the duration of the experiment. These variables included the varying levels of the fungal material in the diet as well as the use of different culture batches (Table 1) containing different levels

of total FB (FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub>), namely MRC 826 BB (5.38 mg FB/kg), MRC 826 B55 (2.61 mg/FB/kg), and MRC BB57 (3.73 mg FB/kg). During the last 8 years only one culture batch (BB57) was used, although the dietary level of the culture material was raised from 0.25 to 0.5% in the low-dose group and from 0.5 to 1% in the high-dose group. The mean total FB intake of the vervet monkeys in the different groups, as a function of the various cultures and dietary levels used during the experimental period, is illustrated in Figure 1.

### Clinical Pathology

For the purpose of this article, the parameters used to assess liver function are presented separately from those associated with kidney function.

**Serum enzymes associated with liver function. General patterns.** Multiple regression analyses of the serum enzyme indicators associated with hepatocellular damage (AST, ALT, LDH, and GGT) indicated that males

**Table 3.** Comparison of body and organ weights of the vervet monkeys fed high and low doses of *F. verticillioides* culture material for different time periods.

Vervet	Terminated (dd/mm/yy)	Body weight		Liver (g)		Left kidney (g)		Right kidney (g)		Total kidney (g)		Heart (g)		Brain (g)	
		(kg)	%	%	%	%	%	%	%	%	%	%	%		
High dose															
681 (F)	30/06/87	3.67	55	1.50	7.564	0.21	7.352	0.20	14.92	0.41	20.5	0.56	71	1.93	
702 (M)	30/08/89	4.29	64.4	1.50	6.84	0.16	7.45	0.17	14.29	0.33	24.7	0.58	69.82	1.63	
705 (M)	24/07/92	4.79	69.2	1.44	8.35	0.17	8.31	0.17	16.66	0.35	36	0.75	63.9	1.33	
688 (F)	03/11/92	2.70	53.5	1.98	8.885	0.33	9.884	0.37	18.77	0.70	16.9	0.63	62	2.30	
709 (F)	14/09/95	3.38	43.62	1.29	8.91	0.26	9.7	0.29	18.61	0.55	21.59	0.64	63.3	1.87	
712 (F)	11/11/98	5.71	79.49	1.39	8.94	0.16	8.11	0.14	17.05	0.30	49.23	0.86	71.96	1.26	
Low dose															
700 (M)	30/10/92	4.94	69.5	1.41	10.2	0.21	9.9	0.20	20.10	0.41	28.2	0.57	65.5	1.33	
696 (M)	14/01/94	5.71	86.96	1.52	10.66	0.19	14.95	0.26	25.61	0.45	39.78	0.70	66.4	1.16	
710 (F)	30/07/97	3.24	82.1	2.53	7.66	0.24	7.37	0.23	15.03	0.46	22	0.68	68	2.10	
711 (F)	11/11/98	4.48	75.78	1.69	9.32	0.21	9.28	0.21	18.60	0.42	31.7	0.71	70.54	1.57	
Controls															
707 (F)	11/11/98	3.23	70.1	2.17	ND		ND		ND		ND		59.88	1.85	
708 (F)	11/11/98	3.69	66.56	1.80	8.43	0.23	8.79	0.24	17.22	0.47	23.9	0.65	57.57	1.56	

ND, not determined.

**Table 4.** Summary of the body weight in relation to the average feed, *F. verticillioides* culture material, and the calculated and estimated fumonisin intake during the experiment.

Vervet	Duration (days)	Body weight (kg)		Average feed intake (g/kg bw/day)	Mean FCM/kg bw/day (mg)	FB/kg bw/day (mg)		Total FB (mg) estimated	
		Initial	Final (dd/mm/yy)			Estimated <sup>a</sup>	Calculated <sup>b</sup>		
High dose									
681 (F)	929	2.57	–	3.07 (30/06/87)	14.12 ± 2.14	80.03 ± 44.59	0.38 ± 0.27 <sup>c</sup>	–	330.4
688 (F)	2,791	2.42	4.05 (24/07/91)	2.70 (03/11/92)	15.45 ± 1.79	90.4 ± 40.9	0.39 ± 0.22	–	861.2
702 (M)	1,630	1.75	4.45 (14/02/89)	4.29 (30/08/89)	14.12 ± 2.14	77.6 ± 38.7	0.36 ± 0.24	–	431.6
705 (M)	2,689	1.76	5.20 (15/03/91)	4.79 (24/07/92)	15.45 ± 1.79	89.5 ± 44.7	0.39 ± 0.22	–	832.7
709 (F)	3,836	1.50	–	3.38 (14/09/95)	16.10 ± 1.55	98.5 ± 55.5	0.42 ± 0.25	–	1714.6
712 (F)	4,990	1.50	3.92 (27/04/95)	3.70 (11/11/98)	16.11 ± 1.50	80.1 ± 35.56	0.35 ± 0.21	0.49 ± 0.20	2147.2
Low dose									
696 (M)	3,228	2.09	6.03 (26/08/93)	5.71 (14/01/94)	14.91 ± 1.21	36.89 ± 18.69	0.15 ± 0.07	–	1304.08
700 (M)	2,747	2.25	6.03 (15/01/91)	4.94 (30/10/92)	14.23 ± 1.46	36.89 ± 18.69	0.15 ± 0.07	–	1022.98
710 (F)	4,527	1.61	3.65 (24/07/91)	3.24 (30/07/97)	15.1 ± 1.08	37.78 ± 20.68	0.16 ± 0.08	–	1846.20
711 (F)	4,990	1.62	–	4.48 (11/11/98)	15.1 ± 1.08	37.78 ± 20.68	0.16 ± 0.08	0.17 ± 0.09	2037.97
Control									
707 (F)	4,990	1.58	3.38 (25/06/96)	3.23 (10/11/98)	17.74 ± 1.67	88.72 ± 8.34	0.01 ± 0.006	–	–
708 (F)	4,990	1.45	3.80 (05/08/98)	3.69 (10/11/98)	17.74 ± 1.67	88.72 ± 8.34	0.01 ± 0.006	0.01 ± 0.01	48.94

<sup>a</sup>Estimated from the feed intake profiles. Levels that were estimated before 1989 were based on the feed intake profiles that were measured during 1989. <sup>b</sup>Dosage was calculated from routine analyses of the stock feed samples since 1992. <sup>c</sup>Exposure of female 681 to 5% for 40 days was not included in calculating the average fumonisin intake profile. The average intake during that period was 3.79 mg FB/kg/day.

showed higher levels of LDH and GGT and females had higher levels of ALT; there was no difference between them with respect to AST serum levels (Table 5; Figure 2). AST and GGT increased significantly as a function of time, whereas ALT and LDH decreased. Females exhibited a higher cholesterol level; males had higher levels of total bilirubin. Overall, the total bilirubin increased as a function of time, but cholesterol was not affected. All the markers mentioned above were significantly ( $p < 0.0001$ ) positively correlated with the levels of the FCM and hence the levels of total FB. Glucose and ALP, serum markers not necessarily associated with liver function, showed a negative ( $r = -0.48$ ;  $p < 0.0001$ ) and a positive ( $r = 0.40$ ;  $p < 0.0001$ ) correlation, respectively, with FCM (Table 5; Figure 2). Glucose tended to increase with time, but

there were no differences between males and females. ALP decreased significantly with age, with males exhibiting higher levels than females. Creatine kinase, measured only from 1991 onward, increased with time and was also positively correlated ( $r = 0.57$ ;  $p < 0.0001$ ) with FCM (Table 5).

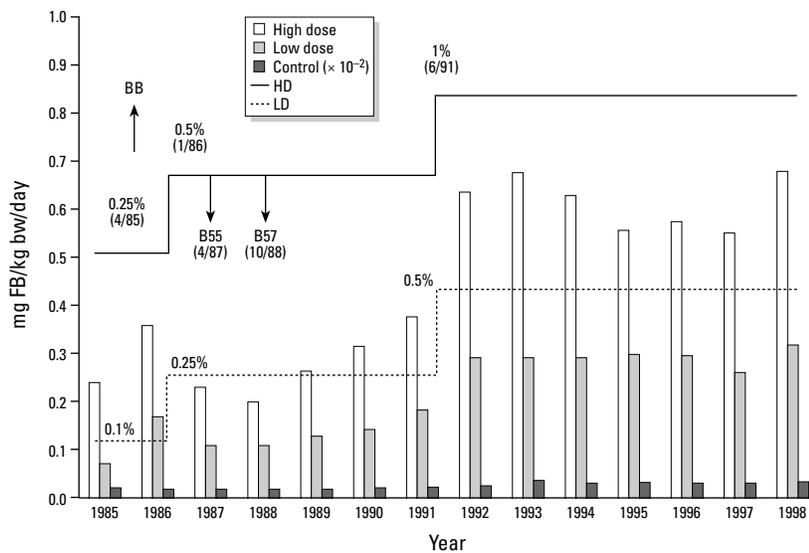
**Dose-response effects.** When considering the above results, two critical time periods exist (Figure 1): when the FB intake was reduced as a result of the use of different culture batches between 1986 and 1990; and when the FB intake profiles were increased due to a doubling of the dietary level of the culture material between 1991 and 1992.

During 1987 a different culture batch of the fungus (B55) replaced the original batch (BB), decreasing the FB intake profile. Both culture batches were included at a dietary

level of 0.5% (high dose) and 0.25% (low dose). The total FB concentrations were 5.38 mg/kg and 2.61 mg/kg culture material for BB and B55, respectively. The FB intake profiles decreased from 0.18 to 0.11 mg/kg bw/day in the low-dose group and from 0.38 to 0.18 mg/kg bw/day in the high-dose group. Toward the end of 1988 another culture batch, B57 (3.73 mg FB/kg), was introduced into the experiment and slightly increased the FB intake profiles to  $0.13 \pm 0.03$  and  $0.29 \pm 0.04$  mg/kg bw/day during 1989 and 1990 for the low-dose and high-dose groups, respectively (Figure 1).

A downward trend occurred in the liver function enzymes, AST and ALT, during the 1986–1988/1989 period in the high-dose group (Figure 2). In the low-dose group, the activities of the enzymes were very similar to those of the control females. No differences were observed between the dosage groups with respect to the total bilirubin and blood glucose levels. However, serum cholesterol levels showed a downward trend in the high-dose group similar to that mentioned for AST and ALT. The levels of ALP tended to decrease with time in the controls and the low-dose group, but this decrease was markedly delayed in the high-dose group. When the dietary level of the culture material (B57) was increased 2-fold during the end of 1991, AST, ALT, and cholesterol levels increased markedly during 1992 in both the high-dose and low-dose groups compared to the control group. There were no clear differences between the low-dose and high-dose groups toward the end of the experiment, although a higher trend was observed initially in AST and cholesterol levels (Figure 2). The same holds for total serum bilirubin and blood glucose, which, respectively, increased and decreased markedly in the high-dose group. The low-dose group also showed marginal effects with levels just above those of the control females. Creatine kinase, monitored from 1991 onward, also showed a marked dose-response effect with no clear-cut difference between the high-dose and low-dose groups (data not shown).

**Serum enzymes associated with kidney function. General patterns.** Serum urea ( $r = 0.41$ ;  $p < 0.0001$ ) and creatinine ( $r = 0.74$ ;  $p < 0.0001$ ) were positively correlated with the dietary levels of FCM and increased significantly compared to the controls (Table 4). In both cases males exhibited higher levels than females; urea decreased and creatinine increased as a function of time. Multiple regression analyses indicated that FCM was negatively correlated ( $r = -0.47$ ;  $p < 0.0001$ ) with a decrease in creatinine clearance. The latter also tended to decrease as a function of time; males had lower clearance values than females.



**Figure 1.** Daily fumonisin intake data of the low-dose (dotted line) and high-dose (solid line) groups of vervet monkeys as a function of the different culture batches of *Fusarium verticillioides* strain MRC 826 (BB, B55, B57) used during the course of the experiment.

**Table 5.** Partial correlation coefficients from multiple regression analyses of different serum parameters associated with liver and kidney function. The correlation factor for FCM was corrected for age and sex.

Dependent	Sex		Age		FCM		$R^2$
	$r^a$	$p$ Level <sup>b</sup>	$r$	$p$ Level <sup>b</sup>	$r$	$p$ Level <sup>b</sup>	
<b>Serum</b>							
Urea (log)	-0.11	0.0035	-0.27	< 0.0001	0.41	< 0.0001	0.2191
Creatinine (log)	-0.40	< 0.0001	0.28	< 0.0001	0.56	< 0.0001	0.3629
Glucose (log)	0.08	0.0744	0.21	< 0.0001	-0.48	< 0.0001	0.2624
Cholesterol (log)	0.17	< 0.0001	-0.05	0.1177	0.40	< 0.0001	0.1856
Total bilirubin (log)	-0.20	< 0.0001	0.27	< 0.0001	0.37	< 0.0001	0.2599
AST (log)	0.00	0.9597	-0.13	0.0001	0.67	< 0.0001	0.4545
ALT (log)	0.24	< 0.0001	-0.19	< 0.0001	0.42	< 0.0001	0.2067
LDH (log)	0.02	0.6535	-0.28	< 0.0001	0.47	< 0.0001	0.2366
GGT (log)	-0.13	0.0002	0.10	0.0055	0.53	< 0.0001	0.3296
ALP (log)	0.14	< 0.0001	-0.72	< 0.0001	0.40	< 0.0001	0.5207
Creatinine kinase (log)	0.00	0.9742	0.29	< 0.0001	0.57	< 0.0001	0.3536
<b>Urine</b>							
Serum creatinine (log) <sup>c</sup>	-0.0125	0.0691	-0.15	0.0371	0.74	< 0.0001	0.5443
Creatinine clearance	-0.0109	0.0308	-0.22	0.0018	-0.47	< 0.0001	0.2541
Urine (log)	-0.0332	0.8618	0.02	0.7994	0.07	0.3917	0.0056

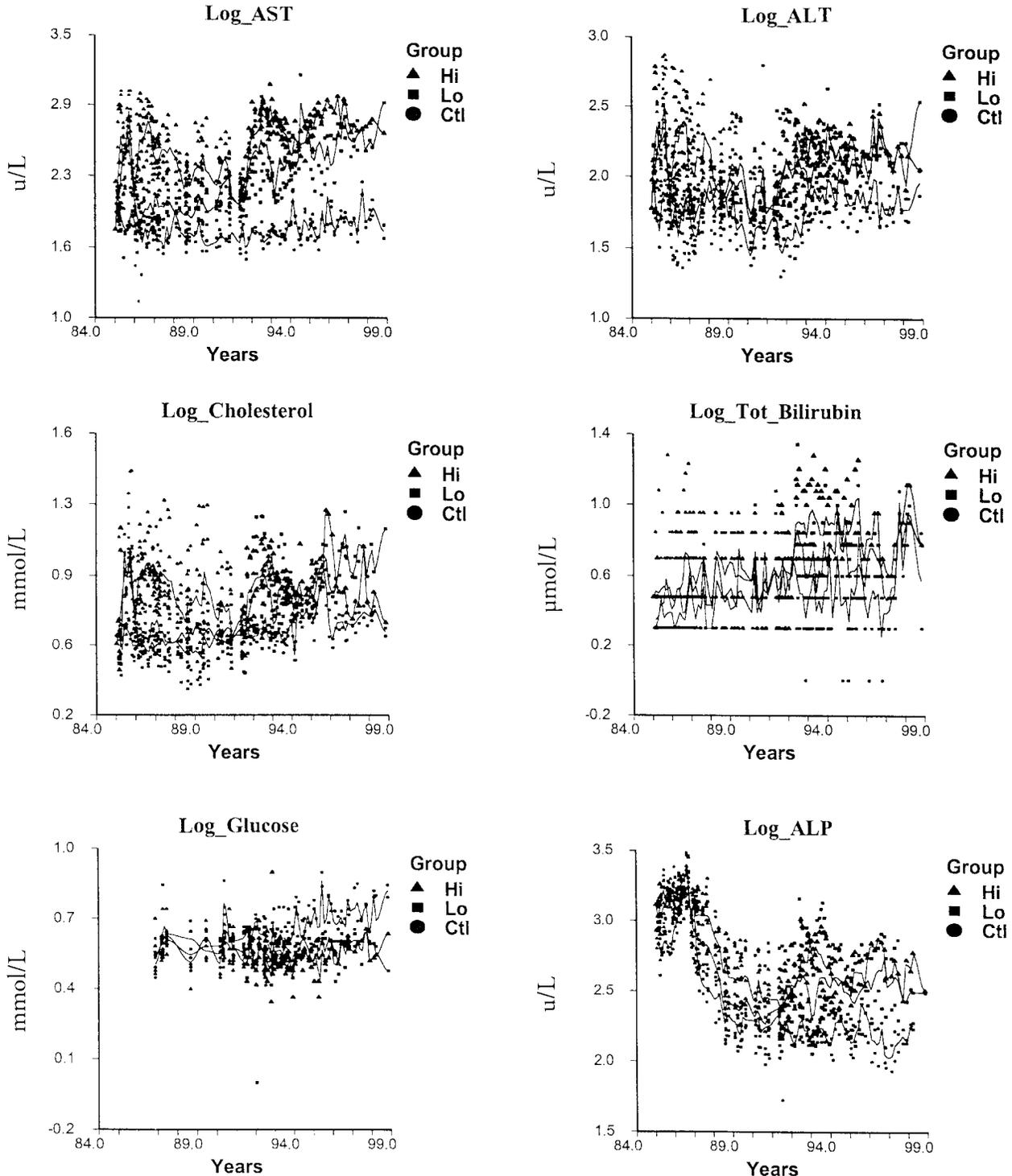
<sup>a</sup>A positive correlation indicates females higher than males. <sup>b</sup>Significance level (on multiple regression analysis). <sup>c</sup>Creatinine clearance was measured from 1991.

**Dose-response effects.** As discussed for the liver function enzymes, two time points were selected: between 1986 and 1988 and between 1991 and 1993 time periods. From 1986 to 1988, no decreases in the serum urea and creatinine levels were noticed in either of the treated groups (Figure 3). During the 1991–1993 dose increase, the serum urea level of the high dose slowly increased until

1995, after which there was a clear distinction between the high-dose and the low-dose and control groups. Creatinine clearance was assessed only from 1992 on, and both the high-dose and low-dose groups showed a reduced capacity (Figure 3). However, the low-dose group tended to revert to control values after about 4 years. The high-dose animals showed a consistently lower creatinine

clearance compared to the control females, and the reduced creatinine clearance capacity relates well with the increased urea and creatinine levels in the serum.

**Differential blood counts.** Differential blood counts were performed from 1991 onward. The white blood cell (WBC) counts were negatively correlated ( $r = -0.49$ ;  $p < 0.0001$ ) with the FCM levels in the diet



**Figure 2.** Multiple regression analyses of selected serum parameters associated with liver function and other important serum components during the experimental period. Hi, high dose; Lo, low dose; Ctl, control; Tot\_Bilirubin, total bilirubin.

(Table 6). In general WBC counts tended to decrease with time, although females exhibited higher WBCs. With respect to the relative contribution to the WBC count, eosinophils, monocytes, and basophils increased as a result of the FCM dietary treatment; the lymphocytes decreased. These parameters were also age dependent; neutrophils and eosinophils tended to decrease and monocytes increased as a function of time. Except for the monocytes, which were higher in males, there were no sex differences in the other WBC components. From the spline smooth (Figure 4), both the low-dose and high-dose groups showed a marked decrease in the WBC count, which tended to approximate values of the controls toward the end of the experiment.

The red blood cell (RBC) count was lower in females ( $r = -0.48$ ;  $p < 0.0001$ ), increased with age ( $r = 0.11$ ;  $p < 0.04$ ), and was negatively correlated ( $r = -0.45$ ;  $p < 0.0001$ ) with FCM dietary levels (Table 6). A similar effect was noticed for hemoglobin (Hb) and the hematocrit (HCT), except that both parameters also increased with age. The red cell distribution width (RDW) positively correlated ( $r = 0.18$ ;  $p < 0.0001$ ) with FCM treatment despite the fact that it generally decreased with age. The graphical plots (spline smooth) indicated that these parameters were affected in both the high-dose and low-dose groups, whereas the low-dose group tended to approximate the control values toward the end of the experiment (Figure 4).

The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were not affected by FCM, whereas the MCV and MCHC were higher in males and tended to increase with age.

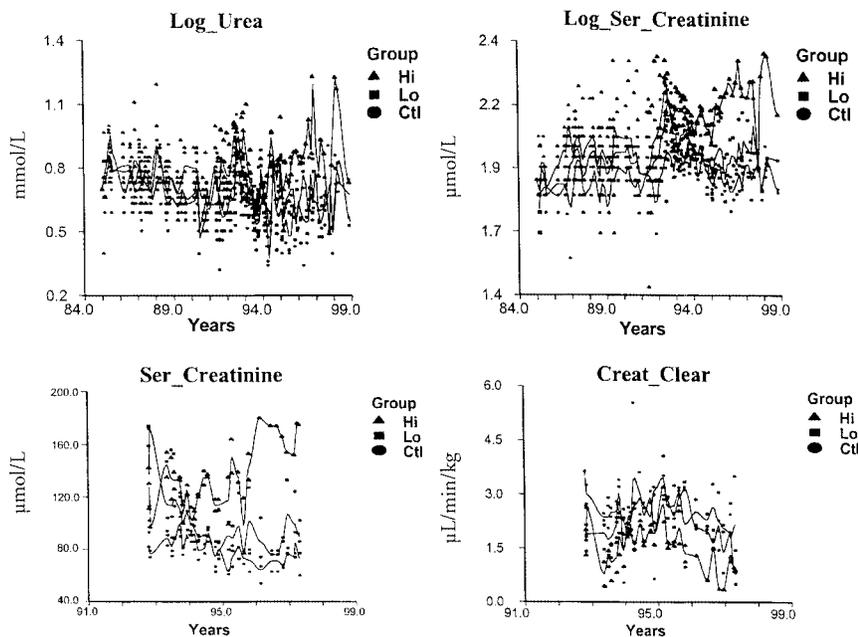
Blood platelet (PL) counts and the mean platelet volume (MPV) significantly ( $p < 0.0001$ ) decreased as a result of FCM treatment despite the fact that PL decreased and MPV increased, respectively, as a function of age. Females had higher platelet counts than males, whereas the MPV tended to be lower in females. The spline smooth graphical plot showed some lowering effects on PL by both the low-dose and high-dose groups throughout the experiment (Figure 4). The MPV clearly showed a decrease in the high-dose group; the low-dose group tended to exhibit similar values toward the end of the experiment.

Two males, one of the high-dose group (male 705) and one of the low-dose group (male 700), were terminated during the second year after the dose increase, followed by the last male (low-dose; male 696) during the third year. One high-dose female (female 688) died within 2 years of the dosage increase.

### Alteration of Sphingolipid Metabolism

During the monitoring period for sphingolipid basis, mean sphingosine levels were not significantly different between any of the experimental groups, whereas sphinganine levels were significantly ( $p < 0.05$ ) elevated in both the low-dose and high-dose groups consuming the FCM (15) as compared to the controls. However, although the high-dose group had a numerically higher sphinganine concentration, the individual variability observed both between and within vervet monkeys in each group caused the 95% confidence limits to be wide. Consequently, this difference between the low-dose and high-dose groups was not statistically significant ( $p > 0.05$ ). During this period, the sphinganine/sphingosine (Sa/So) ratio in the serum increased from 0.43 in the controls to 1.72 in the low-dose and 2.57 in the high-dose groups, with the latter two means significantly elevated over the control ( $p < 0.05$ ) but not significantly different from each other.

During the monitoring period, only a few samples of urine were analyzed. The sphingolipid base levels in urine have been linked to its cellular content because of the presence of exfoliated cells (18); hence, the levels of the individual bases will vary accordingly, making the ratio potentially a more reliable measure of disruption in sphingolipid metabolism. Although the results indicated a numeric increase in the Sa/So ratio for the two experimental groups, once again there was considerable variation and the values were not statistically significantly different.

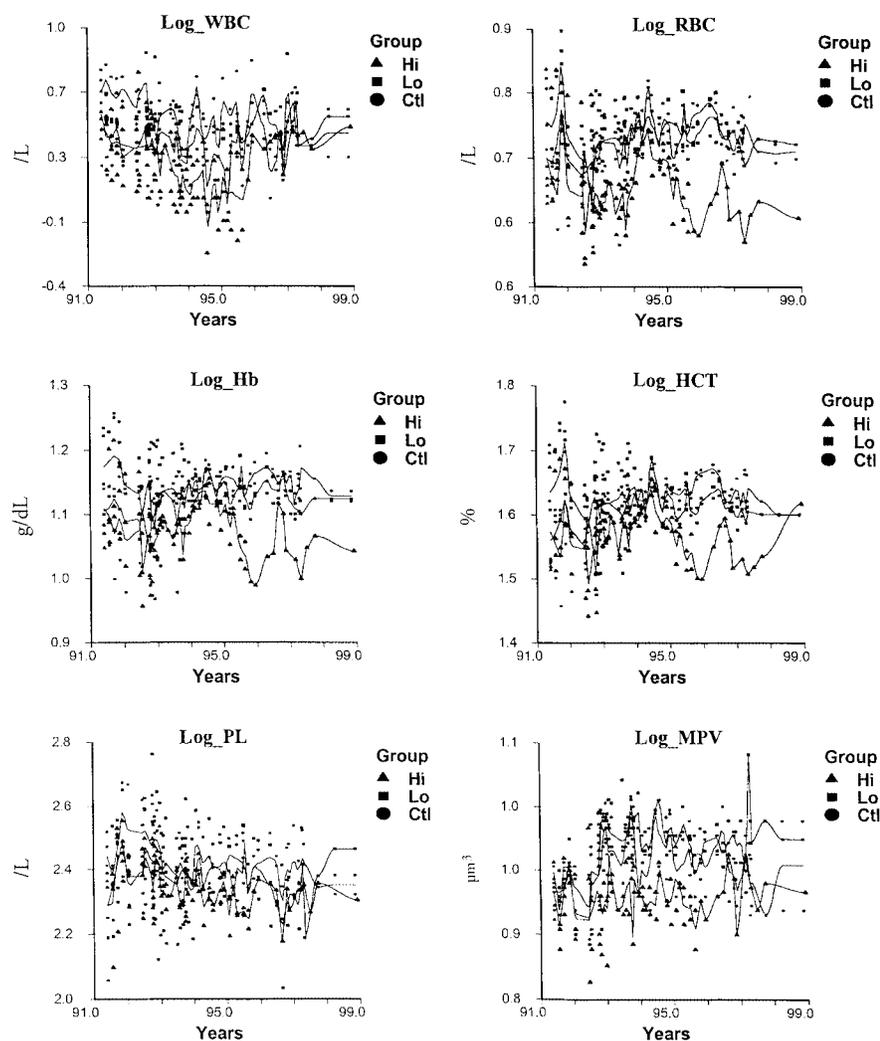


**Figure 3.** Graphical illustration of the effect of the low and high dose of FCM on clinical parameters associated with kidney function. Hi, high dose; Lo, low dose; Ctl, control; Ser\_Creatinine, serum creatinine; Creat\_Clear, creatinine clearance. Creatinine clearance was measured only from 1992 and onward. Serum creatinine measurements were performed during the course of the experiment as well as during the creatinine clearance determinations.

**Table 6.** Partial correlation coefficients from multiple regression analyses of blood parameters measured as a function of sex, year, and FCM. The correlation factor for FCM was corrected for age and sex.

Dependent	Sex		Age		FCM		$R^2$
	$r^a$	$p$ Level <sup>b</sup>	$r$	$p$ Level <sup>b</sup>	$r$	$p$ Level <sup>b</sup>	
Blood parameters							
WBC (log)	0.28	< 0.0001	-0.23	< 0.0001	-0.49	< 0.0001	0.2878
Lymphocytes	-0.03	0.5937	0.06	0.2857	-0.11	0.0435	0.0200
Neutrophils (log)	0.08	0.1941	-0.17	0.0036	-0.08	0.1593	0.0333
Eosinophils (log)	-0.02	0.7484	-0.14	0.0243	0.14	0.0229	0.0469
Basophils (log)	-0.10	0.1213	-0.06	0.3057	0.28	< 0.0001	0.0874
Monocytes	-0.14	0.0216	0.38	< 0.0001	0.44	< 0.0001	0.2725
RBC	-0.48	< 0.0001	0.11	0.0446	-0.45	< 0.0001	0.3603
Hemoglobin	-0.61	< 0.0001	0.20	0.0003	-0.45	< 0.0001	0.4616
RDW (log)	-0.02	0.6688	-0.43	< 0.0001	0.18	< 0.0001	0.2443
MCV (log)	-0.20	0.0003	0.13	0.0232	0.08	0.1606	0.0497
MCH	-0.22	< 0.0001	0.13	0.0216	0.09	0.1224	0.0569
MCHC	-0.05	0.3295	0.02	0.6751	0.04	0.5027	0.0045
PL (log)	0.53	< 0.0001	-0.40	< 0.0001	-0.33	< 0.0001	0.3584
MPV	-0.13	0.0234	0.17	0.0029	-0.35	< 0.0001	0.1631

<sup>a</sup>A positive correlation indicates females higher than males. <sup>b</sup>Significance level (on multiple regression analysis).



**Figure 4.** Dose–response effects of FCM on selected blood parameters from 1991 to 1989. Hi, high dose; Lo, low dose; Ctl, control.

### Histopathology

The liver biopsies, taken at regular intervals up to the middle of 1990, revealed lesions that are characteristic of a chronic toxic hepatitis and that varied from subacute to acute effects in the vervet monkeys receiving the high dose of the culture material (10,11). These changes included perlobular fibrosis, nodular hyperplasia, apoptosis, distortion of reticulin staining pattern, and mild to scant bile duct proliferation, which distorted the normal architecture of the liver. Hepatocyte nodules were uneven in size and showed increased mitosis, uneven nuclear size, and single-cell necrosis (apoptosis). Bi-nucleated cells, fatty changes, and in some cases (female 681) influx of inflammatory cells were noticed randomly throughout the liver. Similar changes were observed in the livers of the vervet monkeys terminated during the experiment. The livers of terminated animals were markedly smaller, with portal-to-portal fibrosis and nodular hyperplasia as constant features (Table 5). In the low-dose group no

specific changes were apparent except in female 710, where disseminated foci of hepatocellular necrosis and inflammatory response occurred with a scant distortion of the reticular pattern. One of the control females (708) also presented with focally disseminated, mild, eosinophilic hepatitis, with no specific toxic lesions of distortion of the lobular structure except in the areas with hepatitis. This lesion was similar to that observed in the low-dose female 710 and, to some extent, in the high-dose female 688 and was ascribed to chronic parasitic hepatitis.

### Discussion

Previous reports on the effects of culture material of *F. verticillioides* MRC 826 on vervet monkeys used in the same long-term experiment (10,11) indicated that the liver is an important target organ. The levels of major serum enzymes used as markers for liver damage (ALT, AST, GGT, and LDH) were elevated in the high-dose and low-dose groups depending on the dietary level of FCM.

Jaskiewicz et al. (10) reported increased levels of AST, ALT, and LDH in vervet monkeys within the first few days in a pilot trial with culture material of MRC 826. The high-dose group that received 1% showed a marked increase within 14 days and a decrease within 2–3 weeks after the dose was lowered to 0.25%. The increase in hepatocellular enzyme activity in the low-dose group was far slower and first appeared 6–7 weeks after the treatment commenced. Increases in total bilirubin and cholesterol paralleled these early changes in liver enzyme activities. These findings were confirmed with multiple regression analysis of all the data as a function of the FCM dietary levels used for the duration of the experiment. The dose–response effects previously described by Jaskiewicz et al. (10) were also clearly illustrated over the period of decreased FCM intake (1986–1988) and the period of increased FCM intake (1991–1993). However, detailed dose–response effects and statistical evaluation of the data with respect to the biochemical parameters measured are complicated by the fact that low numbers of animals were used in each group, both males and females were used, and some of the vervet monkeys from each group were terminated *in extremis* during the course of the experiment. Despite these complications and that fact that some interindividual variations seem to exist among vervet monkeys, the low-dose groups exhibited levels of AST, ALT, LDH, and GGT similar to those of the controls during 1988. During this treatment period, the total FB level was at a minimum and it would appear that at this exposure level (0.10 mg FB/kg bw/day) very few changes are induced in liver function enzymes. The same is true for serum cholesterol, total bilirubin, glucose, and ALP values, although some individual variation appears to occur. Some animals in the high-dose group showed a marked decrease in the levels of the serum enzymes and closely resembled those of the controls. The minimum dosage level of the high-dose group, reached during 1988, was on the order of 0.18 mg FB/kg bw/day.

Changes in the serum parameters, associated with an increase in the FCM level in the diet, were monitored during 1991–1993. All liver function serum enzymes showed a marked increase from the 1991 background level in the high-dose animals. The increased dietary level of FCM represents an FB exposure level from approximately 0.38 mg/kg bw/day in 1991 to 0.64 mg/kg bw/day in 1992. Serum enzyme levels also increased in the low-dose animals after an increase of the FB intake from approximately 0.18 to 0.28 mg/kg bw/day. In some of the low-dose animals the serum enzyme levels equaled those of the high-dose group. A threshold for liver damage in vervet monkeys ranged between

0.10 mg FB/kg bw/day (low dose 1988) and 0.18 mg FB/kg bw/day (low dose 1991), where most of the vervets exhibited baseline values compared to the control females. In general, it appears that males were more sensitive than females because three males were terminated within 2–3 years after the dietary level of the culture material was doubled. Only one high-dose female died during this period.

Histopathologic changes associated with changes in liver enzyme activities have been described in detail. These changes suggest a chronic toxic hepatitis that simulates acute viral hepatitis, with some similarities to the effects induced by aflatoxins. Mild portal-to-portal fibrosis was also described by Fincham et al. (11) in biopsy specimens of both the high-dose and low-dose groups collected up to 1,631 days (middle 1989) after the experiment began. These biopsies also indicated that consumption of culture material of the fungus (high dose) caused atherogenic plasma lipid profiles to develop. These changes entailed elevations of plasma low-density lipoprotein (LDL)-C and apoprotein B strongly associated with accelerated atherosclerosis in vervet monkeys. It was suggested (11) that increased plasma cholesterol could be related to changes at the hepatocyte membrane leading to a reduced or impaired internalization of LDL into the hepatocytes, and hence the reduced clearance from the plasma. The reduced receptor activity might be caused by cytotoxicity induced by the FCM, an aspect that was clearly indicated by the serum enzyme profiles related to liver function. It was suggested that the synthesis of cholesterol was not affected, although in rats cholesterol accumulates both in the plasma and the liver (19). Plaque formation in the aorta of some of the animals sacrificed (Table 3) is therefore of interest in this regard. Additional studies must be conducted to evaluate the role of FB on cholesterol metabolism because it can effect changes in lipid metabolism in rat liver at different levels (19).

We also monitored the effects of the 1986–1988 and 1991–1992 changes in dietary levels of FCM on renal function. The low-dose group did not show any marked changes in serum urea or creatinine during the 1986–1988 culture batch dietary change. However, most of the high-dose animals showed elevated levels and therefore did not reflect the decrease in FB levels from 0.35 mg FB/kg bw/day in 1986 to 0.18 mg/kg bw/day in 1988. The 1991–1992 dosage increase markedly increased the serum creatinine levels in all high-dose vervet monkeys and in most of the animals of the low-dose group. The results of these two time points suggest that a threshold for changes in these two renal parameters exists in the region of 0.18–0.2 mg FB/kg bw/day as discussed for liver function. Creatinine clearance was monitored only

1 year after the dosage increase and showed markedly lower values during 1992 compared to the controls. All females (both high-dose and low-dose groups) showed a remarkable recovery phase over the first 3 years, whereafter creatinine clearance decreased to values similar to those of the control females. Although creatinine clearance is widely interpreted as a measure of glomerular filtration rate (GFR) and therefore renal function, the serum creatinine levels can be affected by many variables apart from glomerular filtration (20). These variables include the reabsorption and secretion of creatinine by renal tubules, which could over- or underestimate the GFR. In the present study, some nephrotoxic effects were noticed in the proximal tubular epithelium that could have decreased the tubular excretion of creatinine and therefore reduced the urinary levels of creatinine and indirectly affected the measured GFR.

Of interest were changes in the level of creatine kinase, which were markedly increased in all the high-dose animals in a dose-dependent manner. Monitoring total creatine kinase activity, measurement normally reflects the muscle isoenzyme because the heart and brain isoenzymes occur at far lower levels (21). As discussed for creatine kinase, care must be taken when interpreting the various clinical biochemical serum parameters. Some of the parameters, such as LDH and ALP, consist of many isoenzymes, each associated with specific tissue compartments, and electrophoretic enzyme characterization is required for identification (20). Bone origin of ALP elevations is quite easy to distinguish from hepatobiliary conditions in the absence of increased levels of AST, ALT, and GGT. AST and ALT have no specific isoenzymes, but AST is quite widely distributed in skeletal and cardiac muscle, liver, and erythrocytes. GGT occurs mainly in the liver and kidneys, but clinically it is confined to liver conditions; in small animals it roughly parallels ALT. Increases in bilirubin can also result from many abnormal physiologic conditions, including liver failure and obstructive biliary disease. Although Jaskiewicz et al. (10) reported cholestasis in the high-dose group, this could not be confirmed in subsequently biopsied animals that received decreased dosage levels of the culture material. It would appear that chronic liver injury was responsible for increased levels of total plasma bilirubin. As discussed for bilirubin, an increase in plasma cholesterol could be related to a number of causes, including diabetes mellitus, liver or biliary disease, or a fatty meal (21). It was suggested that in the absence of cholestasis, a reduced uptake of LDL cholesterol, caused by changes to the hepatocyte membranes, could be an important reason for the increased serum cholesterol (11). Of interest is

the finding of Fincham et al. (11) that culture material caused atherogenic effects in the high-dose vervet monkeys in this experiment.

This study provides interesting exposure parameters to be considered regarding dose–response effects of FB contaminated feeds and/or foods. The no-observed-effect level, with respect to the total fumonisin levels in the corn culture material, for liver and kidney damage in vervet monkeys can be estimated at between 0.11 and 0.18 mg FB/kg bw/day. This level is slightly below the values proposed to effect natural outbreaks of ELEM (0.6–2.1 mg/kg bw/day) (22). The probable daily intake (PDI) calculated for humans consuming corn as the staple diet, such as in the Transkei region of the Eastern Cape Province of South Africa, is 0.36 and 0.047 mg FB/kg bw/day when consuming “moldy” and “healthy” corn, respectively (23). These PDI values decreased considerably when calculated for people consuming corn exported from South Africa (0.002 mg FB/kg bw/day) or from the United States (0.007 mg FB/kg bw/day). Based on the FB-intake profiles of the vervet monkeys of 0.11 to 0.18 mg FB/kg bw/day and an average feed intake profile of  $16.0 \pm 1.6$  mg/kg bw/day, fumonisin contamination levels of corn between 8.21 and 13.25 mg FB/kg diet can be calculated. These values appear not to affect liver and kidney function in the vervet monkey but were suggested to be dangerous to horses, and 10-fold higher values could induce pulmonary edema syndrome in pigs (21). Changes in sphingolipid metabolism, specifically in the sphinganine concentration and the Sa/So ratio, were effected in the serum and also to some extent in the urine of the vervet monkeys at dietary levels of 0.29–0.64 mg FB/kg bw/day. These intake levels represent, as calculated above, contamination levels in corn of 21.7–47.8 mg FB/kg. The vervet monkey appears to be a promising model to study the use of the Sa/So ratio as a biomarker to determine fumonisin exposure in human populations.

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